Remarks

The examiner's further reconsideration of the application is requested in view of the new claims above and further comments which follow below.

Given the examiner's indication that the response filed May 17, 2004 was not fully responsive, the previous claim set has been deleted, and claims 105 - 112 substituted in their places. Claim 105 relates to claim 83, as amended, claim 106 is new, claim 107 corresponds to claim 85, claim 108 corresponds to claim 86, claim 109 corresponds to claim 87, claim 110 is new, claim 111 is an amended version of claim 88, and claim 112 is new.

Claim 105 (amended claim 83) refers to retroviral genes, which should be a sufficient indication that the invention deals with sequences from a retrovirus. Although no sequence is mentioned, the claim points out which retroviral sequences belong to the invention. Reference is made to the GC content, which is a structural feature, and to what extent this GC content can be increased when the codon usage is maximally optimised. The Examiner is submitted to be in error in seeing the invention in a particular sequence. The invention is in the codon usage and the changes to a relevant sequence made in accordance with the technical features of the claim of retroviral genes which have evolved to be expressed at low levels in host cells. This low level expression is a particular essential aspect of viral replication in a host cell. This is a common feature of retroviral genes whose gene product is an enzyme.

Although no reference is made to a nucleotide sequence, the skilled person can easily determine, based on the nucleotide composition, which retroviral genes are suitable for optimalization with the present invention and which have been modified according to the present invention.

The limitation of 53-63 % GC content is not an inherent quality of the sequence, it reflects to what extent has been modified in accordance to the teaching of the present invention.

Summarizing, the claimed subject matter is defined by two important parameters. A wild type gene, that would have a GC content of 66% content after maximal codon optimalization and which has been effectively modified to the extent that the GC content is between 53 and 63, is within the scope of claim 105 (amended claim 83).

New claim 106 introduces an even narrower GC content range between 55 and 61 percent. This range is disclosed e.g. on page 7 line 12.

"retroviral gene" in line 3 of claim 83 on file has been amended to "retroviral wild type gene".

"non preferred codons when referred to the eukaryotic cell" in line 4 of claim 83 on file has been amended to "non-preferred codons when referred to the eukaryotic cell wherein said retroviral gene is expressed."

GC dinucleotide pair refers to GC basepairs, thus the GC content and not to the presence of a GC dinucleotide sequence in the sequence of a gene. The claim language has been amended accordingly. "GC content" is mentioned throughout the application.

The term "detectable" has been omitted from claim 105.

The applicants respectfully disagree that former claim 83 refers to "level of activity". Claims 83 on file refers to "a level [of expressed protein] to provide enzymatic activity. The specification recites that "On the one hand high level expression of a retroviral enzyme will be required to detect the enzymatic activity, on the other hand levels which are too high may cause protein precipitation or cellular toxicity." The amount of protein as such is not a sufficient indication to predict an enzymatic activity. Enzymatic activity is now further explained by referring to the enzymatic activity of retroviral enzymes namely integrase activity reverse transcriptase activity and proteolytic activity. Integrase activity is discussed in extenso in the

examples. Reference to retroviral reverse transcriptase and retroviral protease and their enzymatic activity is found on page 5, lines 15-17.

"The retroviral protein may be a protease, reverse transcriptase, integrase protein or a polyprotein gag-pol precursor thereof. In one embodiment the retroviral protein with enzymatic activity is a lentiviral protein"

Furthermore a reference value has been introduced in claim 105 (amended claim 83) to enhance clarity.

The Examiner points out in the passage from the middle of page 4 to page 6, lines 1-2 that the present invention would only be enabled for the integrase construct of SEQ ID NO:1. The Examiner herein further refers to the prior art which teaches that not all codons should be changed into preferred codons in order to obtain increased expression.

In the present invention it is explained that there exists a certain correlation between the GC content of mammalian gene and a high expression level. Although prior art references (e.g. Seed) mention that not all codons should be optimized there is no teaching to what extent a gene should be optimized in order to obtain increased expression and enzymatic activity. In fact this problem is not even addressed, i.e. the problem with nucleotide pairs and the level of expression. The present invention shows that codon optimization for a number of retroviral genes would result in a GC content above that of highly expressed mammalian genes. For those genes, the invention teaches that codon optimalization should be biased to that specific level encountered in highly expressed mammalian genes. A bias to that specific level for those genes with a high GC content when optimized, has not been suggested in the prior art. It cannot be obvious to make changes to nucleotide sequences and nucleotide pair sequences to solve a technical problem which is not addressed in the prior art. There is no motivation and no expectation of success if there is no motivation.

With this teaching the skilled person has sufficient information to decide whether or not a gene has to be optimized according to the present invention and if so, up to what level this should be done. Assays are available for the testing the enzymatic activity and can be performed in parallel for a large number of transfections with genes being optimized via different alternatives in accordance to the present invention.

The objections on structural proteins in the first complete paragraph of page 6, have become moot after the amendment of claim 83 (now 105) which now refers to pol genes, gag-pol genes and regions thereof encoding retroviral enzymes or parts of retroviral enzymes. Support for "gag-pol" gene is found on page 21 lines 24-31. Support for "part of a retroviral enzyme" is found on the last line of page 4 and the first line of page 5.

With respect to the arguments formulated on page 7, the applicants emphasize that the synthetic gene of SEQ ID NO:1 is a specific example of the broader concept of the invention wherein a synthetic retroviral gene begin expressed in a eukaryotic cell, should not be optimized to its full extent but should be optimized to a specific range of GC content which is encountered in highly expressed mammalian genes, as explained before. This finding provides the link between a structural feature (GC content) and a functional feature (enzymatic activity after expression).

On page 8 the examiner cites a phrase which mentions "An adequate written description of a DNA ... requires a precise definition such as by structure, formula, chemical name or physical properties".

The applicants respectfully submit that the claimed sequences are precisely defined by physical properties, namely C+C content, more a C+C content above 65 % that would have been obtained after complete "optimalization" and a GC content between 53 and 63% that has been obtained after the optimalization according to the present invention. In this context it is also noted that during codon optimalization, the DNA has been modified without changing the amino

acid sequence of the protein being expressed. This further emphasizes that the range of GC is the proper structural feature that will determine protein expression and activity.

With full deference to the above argumentation, new claims 110 and 112 have been introduced to refer to SEQ ID NO:1 and homologues thereof. Support is found for example on page 13, line 28-29.

Comments on claim rejections - 35 USC § 102

The examiner cites US patent 5,786,464 to Seed. This patent deals with env proteins. These proteins are used for the envelope and are without enzymatic activity. An example of such a glycoprotein is gp120, while the present invention claims gag and pol encoded proteins. The Seed patent does refer to retroviral, lentiviral and to HIV viruses in column 2 lines 7-11. The Seed patent also refers, among other things, to the pol protein in column 2 on line 11. The HIV pol protein is cleaved into smaller proteins including integrase. The Seed patent however nowhere refers to HIV enzymes or enzymatic activity in general or to HIV integrase in particular. Nor does it discuss the difficulties with expression of these enzymes. In fact, the reference to these enzymes in Seed is a guess – there is no experimental justification, or otherwise the problems would have been seen. Accordingly, Seed is not enabled for pol proteins.

The examples of the Seed patent only refer to the expression of the viral structural protein gp120 and to the structural rat protein Thy-1. The proteins encoded by the synthetic gene have been identified by immunological analysis, merely indicating that certain epitopes are present on the proteins being expressed by the synthetic gene. Seed does not show that the expressed proteins have a proper structural confirmation. As Seed does not work with enzymes at all, there is no teaching how to modify a retroviral gene encoding an enzyme by codon optimalization whereby during transcription/ translation the proper structure, and in addition, the proper enzymatic activity is preserved.

The specification mentions on page 19, lines 2-5 "An important aspect of the present invention and its applications is the functionality of an expressed retroviral enzymatically active protein, as opposed to mere the high level expression of an enzymatically inactive retroviral protein." The Seed patent only deals with the aspect of high expression and not with the functionality of the expressed retroviral enzymatically active protein. The Seed patent nowhere refers to a specific nucleotide pair frequency, either GC or otherwise, and not all at a specific range of GC content between 53 and 63 % in order to have a GC content that corresponds with that of highly expressed mammalian genes.

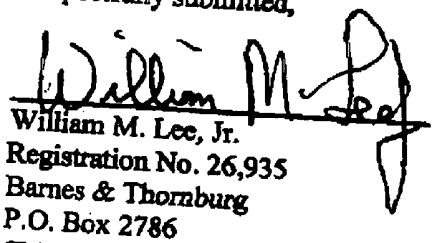
The examiner states the product of Seed has the same structure as those of the present invention, and thus that based on Seed one should arrive at the subject matter as claimed. The use of a small group of host proteins to determine nucleotide pair frequency and the selection of the specific for the GC frequency are not disclosed in the prior art. For the genes in the present claims the GC pair frequency is not disclosed in the prior art. The references to pol and gag genes in the Seed patent are without experimental basis and based on guesswork. There is a "hope" that it might work but no real "expectation".

The present set of claims deals with genes of retroviral enzymes, which have been optimized to specific levels of GC content. The applicants therefore respectfully disagree with the examiner's opinion that the products of Seed have the same structure of the claimed invention and that the products would meet the limitation of detectable enzymatic activity.

Given the above, it is submitted that not only is this response fully responsive to the office action, but also, the claims are in condition for allowance. Such further action by the examiner is solicited.

June 25, 2004

Respectfully submitted,



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